Bi-Layer Wound Dressing System for Combat Casualty Care

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SUMMARY

Burn injuries remain a significant cause of morbidity and mortality during modern military conflicts and peacekeeping operations. Considering that commercially available dressings are not designed to meet the challenges of treating combat burn wounds, DRDC Toronto has designed a novel, absorbent and medicated bi-layer wound dressing to address key requirements for treating external war wounds. In the present report, we assessed our dressing's bactericidal efficacy, wound healing properties, and skin-cooling characteristics using various pre-clinical models. Biopsies taken from full-thickness, pig wounds infected with Ps. aeruginosa and Staph. epidermidis showed a 2- to 5-log reduction in the bacterial load of antiseptic-treated wounds compared to those of control wounds. Though increasing the frequency of dressing changes led to a greater reduction in the wound bacterial load, the contamination levels of all antiseptic-treated wounds remained below 105 CFU/g of wound. Our results also show that 97% of partial-thickness, non-contaminated porcine wounds treated with the DRDC dressing healed within 7 days. In contrast, 92% of the wounds treated with commercial dressings healed within 9 days. Finally, the application of a moist DRDC dressing (to simulate a condition of exudate absorption; DRDCmoist) on a scald burn covering 25% of the dorsal area in rats reduced skin temperature (Tskin) by 1.7oC for 5 min, Tskin in DRDCmoist being comparable to that of control burned rats (BURN) after 25 min. While there were no significant differences between the body temperature (Tip) in BURN and DRDCmoist throughout the 90-min experiment, application of a commercial hydrogel dressing markedly decreased Tip after 90 min (3.03±0.55 oC). These data show that the DRDC dressing is effective in: a) delivering medications, such as an antimicrobial agent, to the wound bed; b) promoting faster healing of the treated wound; and c) providing a transient, but beneficial cooling effect to the skin contact-site, without the adverse effect of inducing whole-body hypothermia.

1.0 INTRODUCTION

Burn injuries have been considered an ubiquitous and potentially life-threatening insult, commonly associated with modern front-line military operations. The risks of burn injury during combat are well recognized, and are unlikely to diminish because of the rapid proliferation of new and powerful thermobaric weapons that target the inherent vulnerability of the soldier to sustain burns [1]. The incidence of accidental burn injuries has also been increasing in recent peacekeeping and training operations due to the mishandling of flammable materials, propellants, chemical agents, munitions, and hot liquids [2, 3]. Military deployment will also expose personnel to accidental burn injuries unrelated to combat (e.g., road traffic accidents, smoking and the use of unguarded flames for cooking, lighting and heating liquids). Clearly, burn injuries can potentially have not only detrimental pathological effects on the soldier but also a tremendous impact on both military and civilian facilities.

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Management of wounds sustained during military operations offers challenges that are different from treating comparable wounds in a civilian setting. First, since delayed evacuation of casualties is not uncommon, immediate wound care may need to be self-administered or facilitated by untrained personnel under extremely challenging and probably hostile environment [4]. Second, the front-line wound care management strategy must also take into consideration that wounds are prone to contaminations and infections in the battlefield environment by soil, organic material, and debris. Data from the Somalia conflict as well as the Falkland Island campaign have shown that approximately 15% to 20% of combat wounds will result in infection [5, 6]. The morbidity and mortality associated with a given wound size may become markedly enhanced if the wound becomes contaminated. Furthermore, it appears that standard field dressings applied on mild to moderate wounds will often slide distally once the casualty resumes normal activities, thereby failing to protect the wounds from further tissue damage and bacterial infection [7]. Reports from medics involved in Operations Enduring Freedom and Iraqi Freedom have also confirmed the emergence of multi-resistant bacteria that do not respond to recommended systemic antibiotic therapies, suggesting the need for a topical antimicrobial treatment to counteract the rapid formation of bacterial biofilms at the wound site. Lastly, another consideration applying specifically to the treatment of burns remain the reduction of the progression of the severity of tissue injury by providing short-term, immediate cooling of the burned skin to prevent further tissue damage [8].

Commercially available dressings are not designed to meet simultaneously the challenges of treating combat burn wounds. DRDC Toronto has therefore designed a novel, absorbent and medicated bi-layer wound dressing to address key requirements for treating external war wounds. The DRDC dressing is composed of a hydrogel wound-contacting layer surimposed by a proprietary polyurethane foam layer. In the present report, we assessed our dressing's bactericidal efficacy, wound healing properties, and skin-cooling characteristics using various pre-clinical models.

2.0 METHODS

2.1 Preparation of wound dressings

The DRDC dressing is composed of a thin (38 mil) hydrogel wound-contacting layer surimposed by a proprietary soft, hydrophilic polyurethane foam layer (10 mm thick; Figure 1). An outer semi-permeable polyurethane membrane can also be added to: allow the controlled release of moisture vapour; provide an effective barrier to water or wound exudate; and, prevent the passage of bacteria through the back of the dressing. All non-medicated dressings were prepared by Avitar Inc. (Canton, MA) under aseptic conditions, and packaged individually in aluminum foil bags (VWR Canlab, Mississauga, ON). Assessment of the sterility of each batch of dressings was performed at DRDC using standard microbiological procedures prior to the application of the samples on wounds.

All medicated DRDC dressings were prepared by immersing for 30 min the material in an aqueous solution containing the antimicrobial agent under study. The drug-loaded dressings were then squeezed under a sterile custom-built benchtop rolling press for 60 s, to retain a standardized amount of the drug solution. Preliminary experiments have shown that the drug is loaded in both layers of the DRDC layers, with the foam layer acting as a drug reservoir (unpublished data).

The protocols of the experimental studies described herein were approved by the institutional animal committee, and conducted in accordance with the guidelines of the Canadian Council on Animal Care.

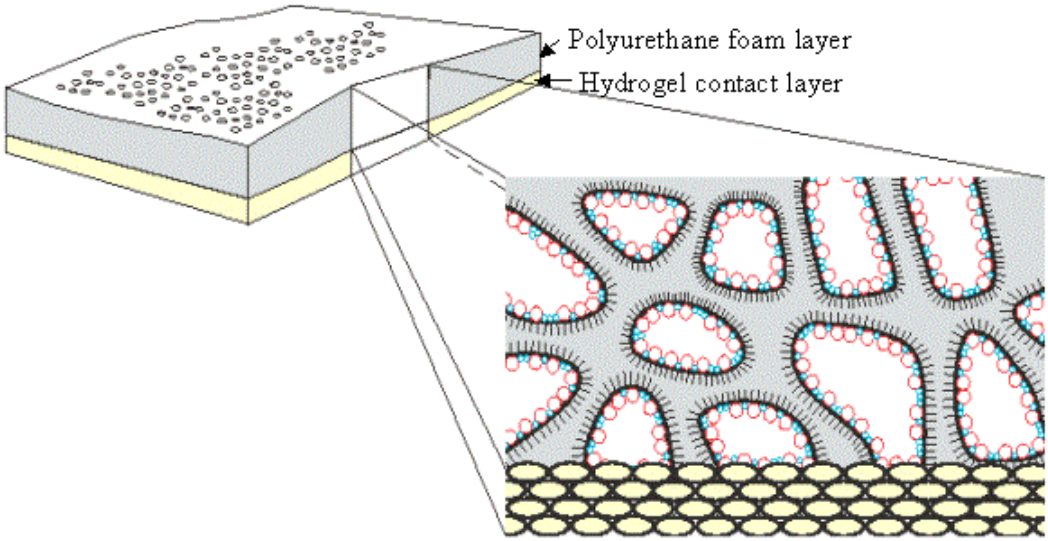

Figure 1. Schematic representation of the DRDC bi-layer wound dressing, consisting of a medicated polyurethane foam over a thin tissue-contacting hydrogel layer to reduce adhesion to the wound. Colored circles represent two types of drug loaded in the pores of the foam layer. For the sake of clarity, no drug was represented in the hydrogel layer.

2.2 Study I: Assessment of *in vivo* Bactericidal Efficacy

Three male Yorkshire pigs (20-25 kg) were allowed to adapt to the environmental conditions (20-25˚C, 12 h light/dark cycle) for at least 7 days before undergoing surgery. Domestic pigs were selected because of the morphological and functional similarities of pigskin with the human skin, and the ability of the pig models to predict wound healing in humans [9]. Animals were housed individually, and had free access to pig chow and water at all times during the study period.

2.2.1 Bacterial Challenge

Clinical isolates of *Ps. aeruginosa*, *Staph. epidermis*, and *Fusobacterium sp*. were used to infect the wounds. The bacterial strains were grown at 37°C in nutrient broth for 18 h in a shaking water bath to obtain a log-phase growth culture. The suspensions were washed 3 times in sterile phosphate-buffered saline (PBS), re-suspended in sterile PBS, and diluted to approximately 10^7 colony forming units (CFU) per mL. Serial dilutions were plated on Pseudomonas Isolation agar (for *Ps. aeruginosa),* Staphylococcus Medium 110 (for *Staph. epidermis*) or Tryptic Soy agar (for *Fusobacterium*) to assess bacterial concentrations in the inoculum. On the experimental day, the three bacterial cultures were mixed together in a ratio approximating 1:1:0.5 (*Pseudomonas*: *Staphylococcus*: *Fusobacterium*; 10^7 CFU in 50 mL).

2.2.2 Experimental Procedures

On the experimental day, each pig was pre-anaesthetized with ketamine (15 mg/kg body weight, i.m.) and acepromazine (0.5 mg/kg body weight, i.m.) followed by gas inhalation (oxygen: 1-2% isoflurane). The dorsal and lateral thorax were clipped, and the skin prepared for wounding by washing with an antibiotic-free soap. Columns of wounds on the dorsum were labelled (using an indelible marker) as A through D, and rows

marked as 1 through 4. Eighteen full-thickness (down to the deep fascia) wounds were created using a 2-cm diameter tissue trephine. Wounds were made 4 cm apart, with columns B & C set 2 cm on each side of the pig's spine. Sterile gauze compresses (Nu Gauze, Johnson & Johnson, New Brunswick, NJ) were applied on the wounds, soaked with a saline/epinephrine solution (1:100 v/v), and allowed to remain *in situ* until complete haemostasis had occurred.

The wounds were loosely packed with 2.5 cm x 2.5 cm sterile gauzes, and inoculated with 3 mL of the bacterial suspension. The wounds were then covered for 20 min with an occlusive sterile film (Saran Wrap®, SC Johnson, Racine, WI) to prevent drying. At the end of the infection procedure, the gauzes were removed, and the wounds were each dressed with a 2-cm DRDC bi-layer dressing containing either 1 mL of PBS (Control), or were loaded in aqueous solutions of 0.5 % chlorexhidine digluconate or 1 % chloramphenicol, as described previously. Each experimental dressing was then covered with a piece of sterile parafilm. Layers of adhesive PVC tape (Elastoplast™, Smith & Nephew, Lachine, QC) were then applied to hold down firmly the experimental dressings. The entire trunk of the pig was then wrapped with a layer of elastic self-adhesive bandage (Elastoplast™, Smith & Nephew, Lachine, QC). A dose of narcotic (i.m. buprenorphine, 0.1 mg/kg body weight) was administered and a Duragesic® patch (Janssen Pharmaceutica, Titusville, NJ**)** delivering 50 μg/h fentanyl for 72 hours was then applied prior to returning the animal to its pen.

Dressings were replaced daily or every other day during the 4-d study (n=6 per experimental group per dressing change). A 4-mm biopsy was taken at the centre of 6 wounds in each experimental group at each dressing change. Tissues were placed into pre-weighed tubes, homogenized in cold PBS, and plated serially on SM110 and PIA to determine the bacterial counts. Animals were humanely euthanized at the end of the 4-d study period using T-61 (i.v.; Hoechst-Roussel Canada Ltd, Montreal, QC) following sedation (ketamine, 15 mg/kg body weight; acepromazine, 0.5 mg/kg body weight, i.m.).

2.3 Study II: Assessment of Wound Healing Properties

2.3.1 Surgical Procedures

On the experimental day, 5 male Yorkshire pigs (20-25 kg) were anaesthetized, and their skin prepared for wounding as previously described. Seventy partial-thickness (0.4 mm deep) 1-cm^2 wounds (five columns of seven wounds on each side of the pig's spine) were marked using an indelible pen on the back of the animal with a custom-built Lexan® template. The wounds were then individually created, 1 cm apart, at the marked location using a dermatome (Tyler Research Corp., Edmonton, AB). Sterile gauze compresses were applied on the wounds, soaked with a saline/epinephrine solution (1:100 v/v), and allowed to remain *in situ* until complete haemostasis had occurred. Groups of 5 adjacent wounds were then randomly covered with strips (2.5 cm x 15 cm) of sterile, non-medicated DRDC tri-layer dressing or Allevyn® (Smith & Nephew, Lachine, QC) (n=125 wounds per experimental group). Layers of adhesive PVC tape were then applied to the back of the pigs to hold down firmly the experimental dressings. The trunk of the pig was finally wrapped with a layer of elastic self-adhesive bandage. No experimental or securing dressings were applied to the last row of wounds on each pig (air-exposed control; n=100 wounds). Analgesia was administered as previously described.

2.3.2 Experimental Procedures

Since wound healing in this model rarely occurs within the first 4 days after wounding [10], the following procedures were performed daily from day 5 to day 9 only. Briefly, pigs were anaesthetized and all

dressings were removed. Rows of wounds were harvested in one strip using a 2 cm x 2 cm blade set at 0.8 mm depth, according to a pre-determined harvesting protocol (maximum 15 wounds per pig per day). The wound strips were placed in a sodium bromide solution for 24 hours to provide an assessment of the extent of wound healing. A new experimental dressing was then applied to all wounds that had not been harvested, and the animal was returned to its pen.

Following sodium bromide incubation, dermal and epidermal layers were separated, and the presence of holes in the epidermal sheet was noted. Epidermal sheets harvested intact and not demonstrating any defects were scored as being completely healed; any defects was scored as not being healed. Animals were humanely euthanized at the end of the 9-d study period as previously described.

2.4 Study III: Assessment of Skin Cooling Properties

Forty male, Sprague-Dawley rats (250-275 g) were allowed to adapt to the environmental conditions (22^{o}C, 12 h light/dark cycle) for at least 7 days before undergoing burn injury. Animals were housed in groups of 5 until the injury, and individually thereafter. They had free access to food and water at all times during the study period.

2.4.1 Burn Injury Protocol

Our rodent model of scald injury was adapted from Walker et al. [11] and Martineau et al. [12]. Briefly, rats were anaesthetized (2.0% halothane:N_2O:O_2), and received analgesics (buprenorphine, 50 ug/kg body weight; s.c.). Their back was then clipped, depilated, and cleansed using standard procedures. A 25 percent total body surface area full-thickness scald of the dorsum was produced under anaesthesia by placing the animal in a custom-built Teflon® template, and dipping the clipped, exposed dorsum in water at 90°C for 10 s. Sham-burned rats (SHAM) were immersed in warm (37°C) water for the same time interval. The rats were then rapidly removed from the restraining device, and their skin pat-dried for 5 s with a sterile gauze pad. All animals received a subcutaneous injection of sterile saline.

2.4.2 Experimental Procedures

Immediately following completion of the burn injury procedures, one of 4 small thermistors threaded into a sterile stainless steel tether was inserted 2 cm into the abdominal cavity through a small incision (5 mm), 1 cm below the edge of the burn wound. The thermistor was maintained in place with a nylon non-absorbable suture (Johnson & Johnson, New Brunswick, NJ) threaded through a loop made in the thermistor. The incision was closed in 2 layers with non-absorbable interrupted sutures. The remaining 3 thermistors were sutured 2.5 cm apart on the dorsal burned skin, 1 cm lateral to the spine. The first probe was positioned approximately 2 cm distal to the nape of the neck. The second and third probes were randomly located on alternate sides of the spine on each animal.

The entire burn wound in 3 experimental groups (n=8 per group) was then covered with a hydrogel dressing (2nd Skin®, Spenco Medical (UK) Inc., Wako, TX), a standard, tri-layer DRDC dressing (5 mm thick; 5 cm x 10 cm; $DRDC_{dry}$), or one hydrated to 50% of its maximum absorption capacity with warm water ($DRDC_{moist}$). The latter group simulated a condition of high exudation from the wound. The experimental dressings were applied within 20 min following the burn injury; this time interval was selected based on the estimated delay in attending to a burn wound sustained in the battlefield. A single layer of a 6-cm wide self-adherent non-woven wrap (Coban™, 3M, London, ON) was then used to further secure in place the

experimental dressings (i.e., 2nd Skin; $DRDC_{dry}$, and $DRDC_{moist}$), and to cover the untreated burn (BURN) or sham burn (SHAM). The animals were returned to their cage, the tether being positioned well out of reach of the animals. Temperature data were then acquired for 90 min using a small data logger secured to the cage lid. All rats were euthanized by cervical dislocation (under general anaesthesia) once the data acquisition was completed.

2.5 Statistical Analysis

Statistical analyses were completed using Statistica (Version 6.1, Statsoft, Inc.). In all studies, a two-way analysis of variance (ANOVA) for repeated measures with two within-subject variables (frequency of dressing change or time elapsed since application of dressing; type of wound dressing) was used to determine statistical significance in: bacterial counts in the wounds (Study I); wound healing (Study II); and, intra-abdominal and skin temperature (Study III).

For all analyses, p-values were calculated using the Greenhouse-Geisser epsilon correction for repeated measures. When statistical significance was determined for main or interaction effects, a Neumann-Keuls post-hoc analysis was completed to locate significant differences. Significance was deemed to exist when $p<0.05$.

3.0 RESULTS

3.1 Study I: Assessment of *in vivo* Bactericidal Efficacy

All animals survived for the duration of the experiment. The DRDC dressings were easily removed from the wounds, no residual layer of gel being left behind. The effect of daily wound dressing changes on *Ps. aeruginosa* and *Staph. epidermidis* growth in infected, full-thickness porcine wounds are shown in Figures 2 and 3. *Ps. aeruginosa* and *Staph. epidermidis* bacterial counts in the control wounds increased to 1.10×10^6 CFU/g and 1.03×10^8 CFU/g within 48 h, respectively, these levels plateauing for the next 48 h (Figures 2 and 3). In contrast, biopsies taken from the wounds showed a 2- to 5-log reduction in the bacterial load of antiseptic-treated wounds compared to those of control wounds. *Ps. aeruginosa* counts in the wounds treated with chloramphenicol-loaded dressings were 2-log lower for the first 24 h than when applying the chlorhexidine-loaded dressings (Figure 2). However, there was no statistical difference in the *Ps. aeruginosa* counts in the wounds on day 4, following daily changes of either antiseptic-loaded dressings. Interestingly, chlorhexidine showed a 75% greater bactericidal efficacy against *Staph. epidermidis* than chloramphenicol did throughout the study (Figure 3). Nevertheless, daily application of the antiseptic-loaded DRDC dressings maintained the wound bacterial load below 10^4 CFU/g for both bacteria throughout the 4-d study.

The effect of altering the frequency of dressing changes on *Ps. aeruginosa* and *Staph. epidermidis* growths on day 4 in infected, full-thickness porcine wounds is shown in Figures 4 and 5. Increasing the frequency of dressing changes led to a significantly greater reduction ($p<0.05$) in the number of viable bacteria in the wounds. However, this bactericidal effect was significantly greater for *Staph. epidermidis* than for *Ps. aeruginosa*. It is noteworthy that the contamination levels of all antimicrobial-treated wounds remained below 10^5 CFU/g of wound, irrespective of both the drug loaded in the wound dressing and frequency of wound dressing change.

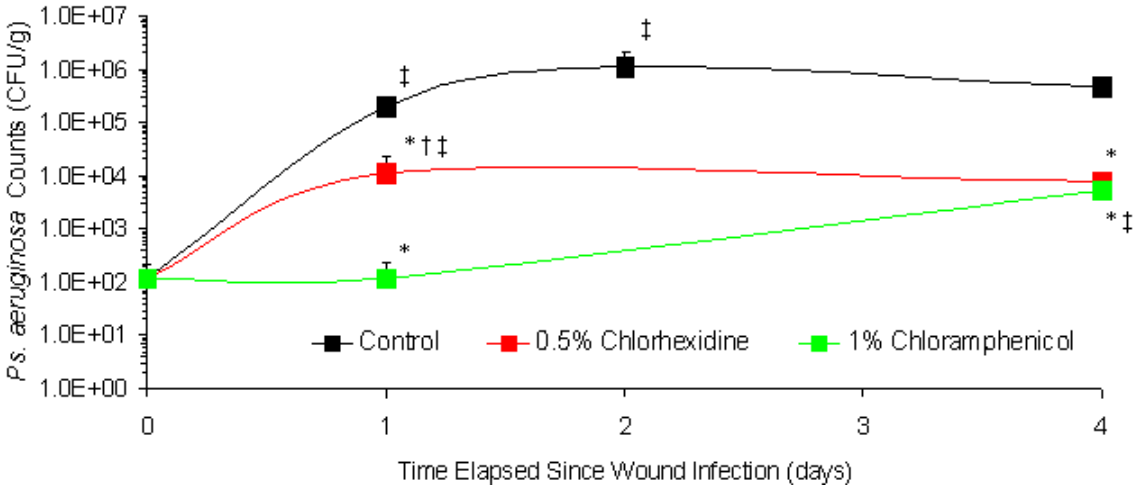

Figure 2. Effect of daily wound dressing changes on *Ps. aeruginosa* growth in porcine full-thickness wounds contaminated with an inoculum containing 10^7 CFU *Ps. aeruginosa, Staph. epidermidis, and Fusobacterium* sp. (1:1:0.5). Wounds were treated with a drug-free DRDC dressing or one containing either 0.5 % chlorhexidine or 1% chloramphenicol. Data are expressed as means ± standard error of the mean (SEM; n=6). * Significantly different from control ($p<0.05$) † Significantly different from chloramphenicol ($p<0.05$). ‡ Significantly different from previous time interval ($p<0.05$).

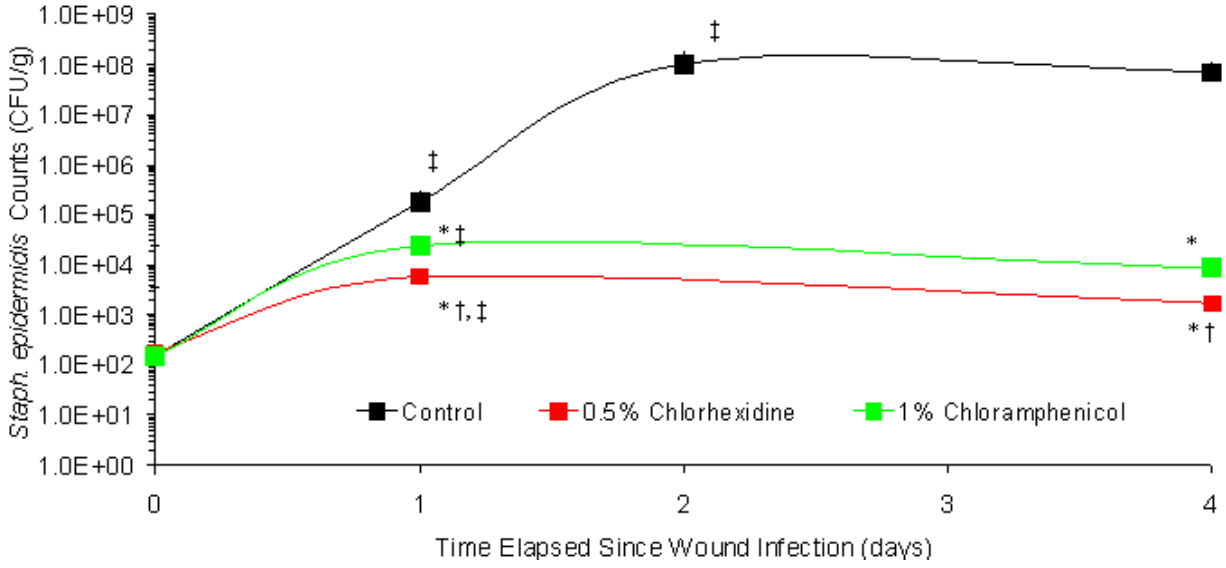

Figure 3. Effect of daily wound dressing changes on *Staph. epidermidis* growth in porcine full-thickness wounds contaminated with an inoculum containing 10^7 CFU *Ps. aeruginosa, Staph. epidermidis, and Fusobacterium* sp. (1:1:0.5). Wounds were treated with a drug-free DRDC dressing or one containing either 0.5 % chlorhexidine or 1% chloramphenicol. Data are expressed as means ± SEM (n=6). * Significantly different from control ($p<0.05$) † Significantly different from chloramphenicol ($p<0.05$) ‡ Significantly different from previous time interval ($p<0.05$).

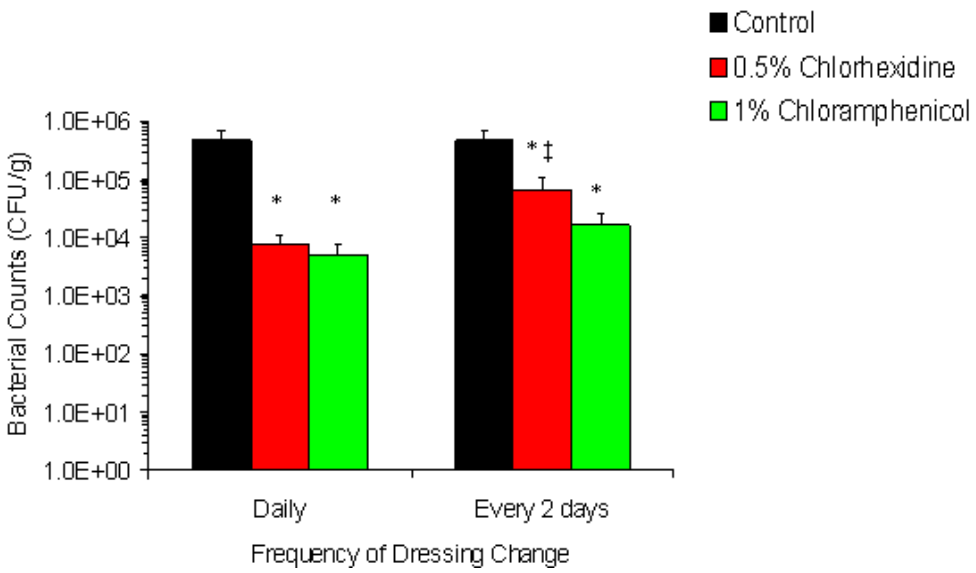

Figure 4. Effect of altering the frequency of dressing changes on *Ps. aeruginosa* growth at day 4 in porcine full-thickness wounds contaminated with *Ps. aeruginosa, Staph. epidermidis, and Fusobacterium* sp. Wounds were treated with a DRDC dressing containing no drug, 0.5 % chlorhexidine or 1% chloramphenicol. Data are expressed as means ± SEM (n=6). * Significantly different from control ($p<0.05$) ‡ Significantly different from daily dressing change ($p<0.05$).

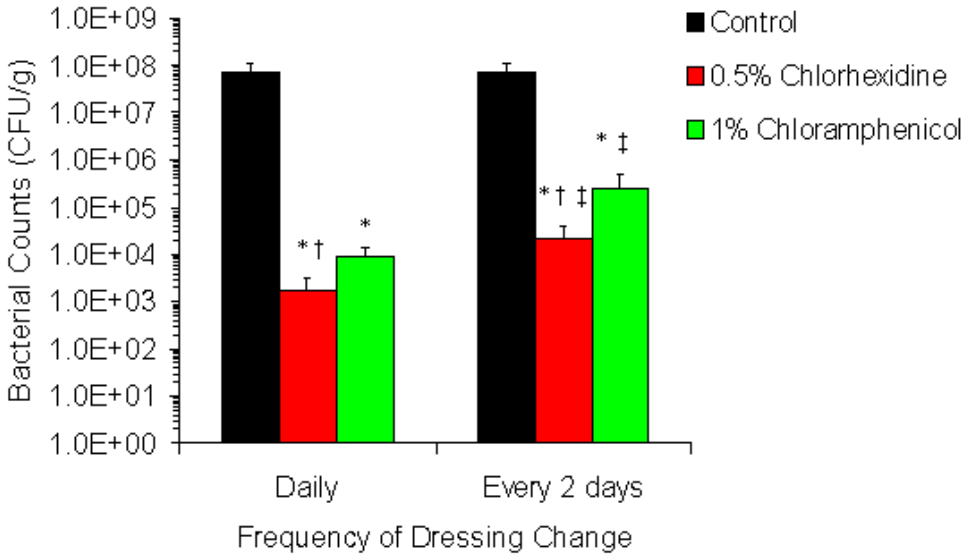

Figure 5. Effect of altering the frequency of dressing changes on *Staph. epidermidis* growth at day 4 in porcine full-thickness wounds contaminated with *Ps. aeruginosa, Staph. epidermidis, and Fusobacterium* sp. Wounds were treated with a DRDC dressing containing no drug, 0.5 % chlorhexidine or 1% chloramphenicol. Data are expressed as means ± SEM (n=6). * Significantly different from control ($p<0.05$) † Significantly different from chloramphenicol ($p<0.05$) ‡ Significantly different from daily dressing change ($p<0.05$).

3.2 Study II: Assessment of Wound Healing Properties

All animals survived for the duration of the experiment. Since the experimental dressings remained securely fastened, all wounds were therefore included in the calculations. Figure 6 depicts the number of non-contaminated, partial-thickness wounds healed over a 9-d period when applying daily either a non-medicated DRDC dressing or Allevyn®. It is noteworthy that the wounds healed poorly when left air-exposed for the duration of the study, with only 25% of them healed after 9 days. In contrast, 36% of the wounds covered with the DRDC dressing had healed after 5 days, this healing rate being 4.5 times greater ($p<0.05$) than that observed for the Allevyn®-covered wounds. While the wounds covered with Allevyn® thereafter healed faster for the next two days, the number of wounds healed remained significantly higher ($p<0.05$) in the DRDC dressing group for 7 days (Figure 6). No further increase in the number of partial-thickness wounds healed was observed in either experimental group for the remainder of the study. However, our results show that while 97% of the partial-thickness wounds treated with the DRDC dressings healed within 7 days, a comparable value was only reached after 9 days when covering the wounds with Allevyn®. While all DRDC dressings were not or minimally adherent to the wounds, 20% of the Allevyn® dressings were embedded in the wound bed at day 5.

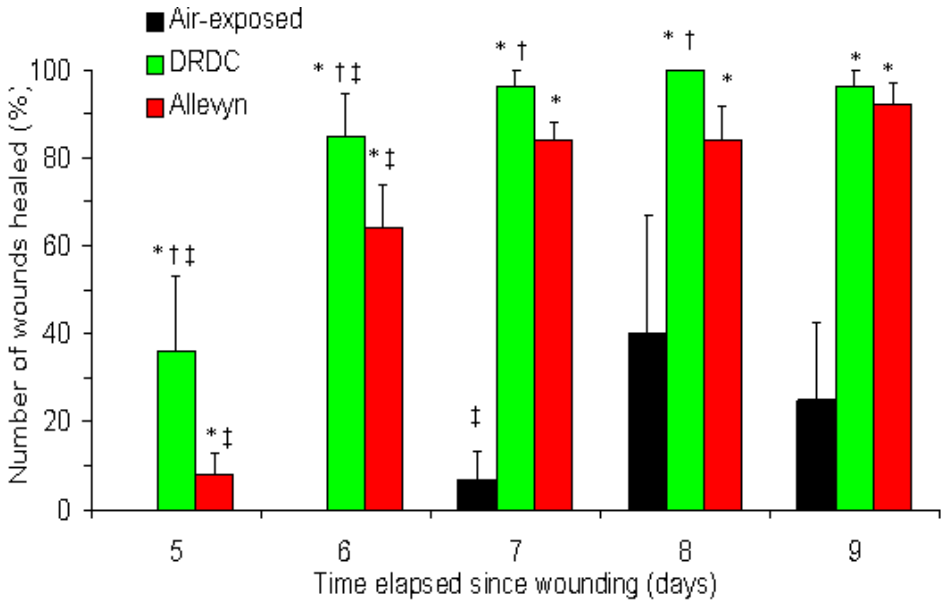

Figure 6. Kinetics of wound healing in a porcine model of partial-thickness, non-contaminated wounds when applying daily either a non-medicated DRDC dressing or Allevyn® (n=25 per time point per group). Control wounds were left air-exposed (n=20 per time point). Data are expressed as means ± SEM. * Significantly different from air-exposed ($p<0.05$) † Significantly different from Allevyn® ($p<0.05$) ‡ Significantly different from previous time interval ($p<0.05$).

3.3 Study III: Assessment of Skin Cooling Properties

All animals survived for the duration of the experiment. There were no significant differences in the skin temperature (T_{skin}) between the 3 dorsal sites recorded for any of the rats; the recorded T_{skin} of all sites were therefore used for comparing the different experimental treatments. Figures 7 and 8 depict the changes

in T_{skin} and intra-abdominal temperature (T_{ip}) over a 90-min application of hydrogel dressings (2nd Skin®) or DRDC dressings (dry or moist) on burn wounds covering 25% of the total body surface area in rats. Burn injury increased T_{skin} by 0.50±0.20 °C within 5 min ($p<0.05$), T_{skin} slowly increasing by 0.78±0.25 °C above the pre-burn injury value for the remainder of the study. T_{ip} in BURN decreased by 1.36±0.27 °C within 20 min of the burn injury, T_{ip} plateauing for the remainder of the study (Figure 8). This reduction in T_{ip} while observing a raise in T_{skin} can likely be explained by a reduction in the level of activity of the rat due to the burn injury.

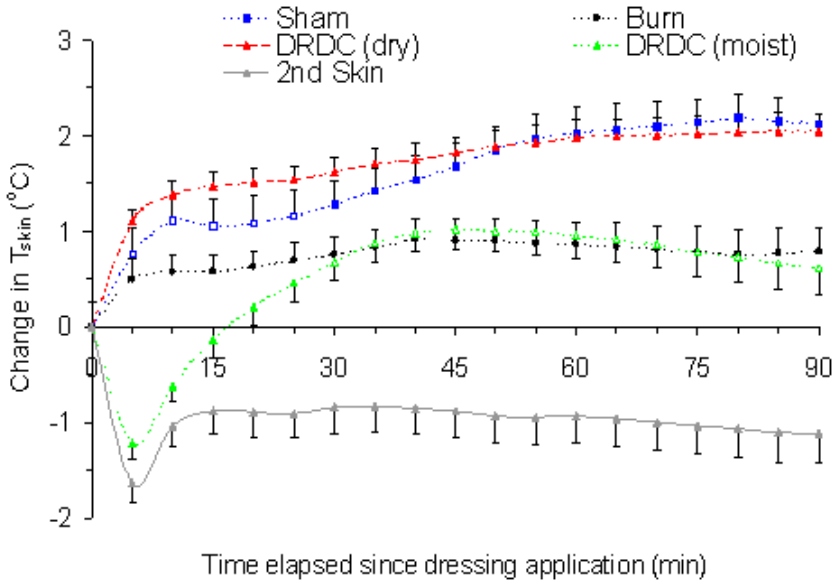

Figure 7. Changes in skin temperature (T_{skin}) when applying hydrogel dressings (2nd Skin) or DRDC dressings (either dry or moist) for 90 min on burn wounds covering 25% of the total body surface area in rats. The animals in the SHAM and BURN groups were dressed with one layer of the secondary dressing only. Data are expressed as means ± SEM (n=24). Filled symbols indicate a difference from BURN ($p<0.05$). Dashed lines indicate significantly different from 2nd Skin ($p<0.05$). DRDC (moist) was different from SHAM and DRDC (dry) at all time intervals ($p<0.05$). There were no significant differences between DRDC (dry) and SHAM throughout the experiment.

Application of a dry DRDC dressing ($DRDC_{dry}$) to burn wounds for 10 min further increased T_{skin} by 0.60 °C compared to that of BURN ($p<0.05$), this increase in T_{skin} doubling by 90 min. Furthermore, $DRDC_{dry}$ prevented the burn-induced reduction in T_{ip} throughout the study (Figure 8). Interestingly, there was no statistical difference in T_{ip} or T_{skin} between SHAM and $DRDC_{dry}$ throughout the study period, suggesting that the polyurethane foam layer did not act as an insulator. In contrast, the application of a moist DRDC dressing (to simulate a condition of exudate absorption) reduced T_{skin} by 1.7°C for 5 min ($p<0.05$) compared to that of BURN. A greater reduction in T_{skin} was observed following application of the hydrogel dressing compared to that of $DRDC_{moist}$ ($p<0.05$). While T_{skin} in $DRDC_{moist}$ and BURN were comparable within 25 min, T_{skin} remained 1.5 °C lower following application of the hydrogel dressing throughout the 90-min experiment compared to that of BURN. While there were no significant differences between T_{ip} in BURN and $DRDC_{moist}$ throughout the experiment, application of a hydrogel dressing markedly decreased T_{ip} after 90 min (3.03±0.55 °C).

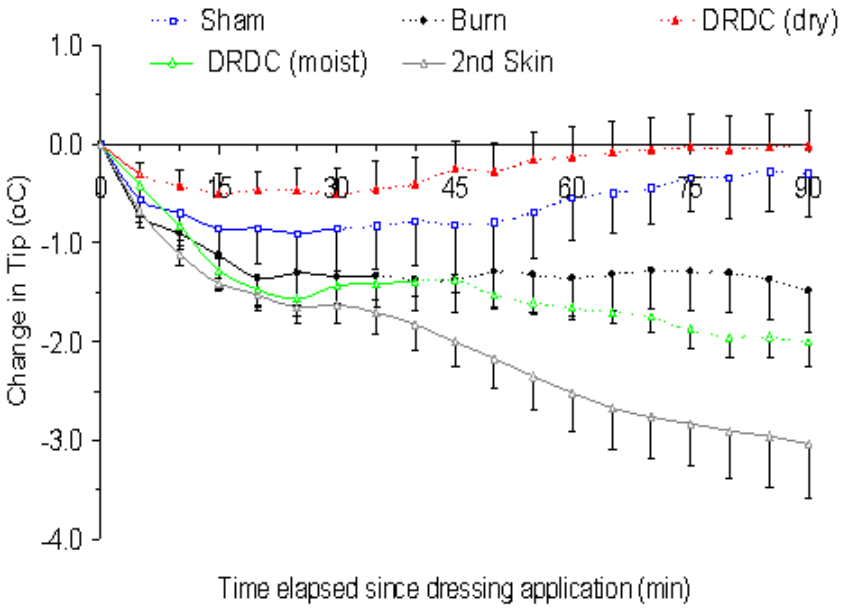

Figure 8. Changes in intra-abdominal temperature (T_{ip}) when applying hydrogel dressings (2nd Skin) or DRDC dressings (either dry or moist) for 90 min on burn wounds covering 25% of the total body surface area in rats. The animals in the SHAM and BURN groups were dressed with one layer of the secondary dressing only. Data are expressed as means ± SEM (n=8). Filled symbols indicate a difference from BURN ($p<0.05$). Dashed lines indicate significantly different from 2nd Skin ($p<0.05$). DRDC (moist) was different from SHAM for the last 30 min only ($p<0.05$). DRDC (moist) was different from DRDC (dry) after 10 min ($p<0.05$). There were no significant differences between DRDC (dry) and SHAM throughout the experiment.

4.0 DISCUSSION

Several new types of wound dressings have been commercialised in recent years, as it is accepted that designing an ideal wound dressing that will simultaneously meet all of the clinical requirements for treating the various types of wounds is impossible. Our data show that we have designed a layered wound dressing that will meet the unique challenges of treating combat wounds. Indeed, the combination of the foam and hydrogel layers: facilitates the reduction of bacterial load of wounds through incorporation of therapeutic agents; protects the wounds from additional trauma and contamination; absorbs wound exudates while maintaining a moist environment; is minimally adherent; promotes wound healing; and, provides surface cooling without inducing significant whole-body hypothermia. These properties make our dressing an excellent wound care management for the treatment of wounds sustained during military operations, especially burn wounds.

The types of bacteria used in the present studies represent common wound pathogens. While *Ps. aeruginosa* remains the most common and dangerous pathogen on burn injuries, the presence of *Staph. epidermidis* strains has also increased in many types of wounds, due to their prevalence on human skin, their ability to adhere to biomaterials, and a greater resistance to most antibiotics [13]. Bacteria can colonize unprotected burn wounds within 12-24 h, with microbial levels reaching 10^8 microbes per g tissue within 48 [14]. Furthermore, conventional dressings are susceptible to strikethrough of wound fluid, thereby providing a route for the bacteria into the wounds [15]. Although suppression of skin flora with an appropriate topical antiseptic agent kills bacteria, the suppressed microflora can rapidly grow back, and invade the compromised

wound site. Furthermore, therapeutic agents applied to the wounds will undergo inactivation by body fluids and other organic debris. It is therefore not surprising that the early application of medicated wound dressings that continuously deliver antiseptic to the wounds remains the most effective way for preventing invasive wound infection.

Selection of the ideal antiseptic for treating contaminated wounds remains a controversial decision. Chlorhexidine and chloramphenicol were loaded in our dressings due to their broad-spectrum antimicrobial activity, and their ability to maintain a local residual antibacterial activity for hours without being neutralized by blood and wound exudates [16, 17]. However, late onset hypersensitivity to chlorhexidine has been observed following its application on both intact skin and wounds [18]. Furthermore, topical application of chlorhexidine has been shown to delay wound healing at concentrations above 0.1% [19], partly due to its cytotoxicity to newly formed keratinocytes [20], and its immunosuppressive effects on exposed macrophages [21]. Interestingly, irrigation of corneal abrasions with 1% chlorhexidine did not impair the rate of re-epitheliazation compared to that of control wounds [22]. Although we used a 0.5% chlorhexidine solution to load the drug into our dressings, the amount that was effectively incorporated during these procedures was likely much lower. Indeed, Loke et al. [23] have shown that the amount of chlorhexidine gluconate incorporated in their carboxymethyl-chitin hydrogel material was 3 to 8 times less than the loading concentration used. The magnitude of this relationship is likely to be material-specific, as we have previously shown a two-fold reduction in the amount of ciprofloxacin loaded into a proprietary hydrogel compared to the loading solution [24]. The amount of chlorhexidine incorporated into our dressings was sufficient to maintain the bioburden below the 10^5 CFU/g for the duration of the experiment, a bacterial threshold that has been shown to allow wound healing to proceed uneventfully, despite the wound bacterial colonization [25]. Thus, it appears that bacteria remain more detrimental to wounds and wound healing than the perceived injurious effects of antiseptic agents, which may explain that chlorhexidine is included in many commercial wound care products such as Bactigras™ (Smith & Nephew), Serotulle™ (Leo Laboratories, Ltd.) and BioPatch™ (Johnson & Johnson). Nevertheless, we are currently undergoing studies to investigate the usefulness of other antiseptic agents with lesser side effects on wound healing.

Our finding of comparable bactericidal efficacies of chlorhexidine-loaded wound dressings against both *Ps. aeruginosa* than *Staph. epidermidis* is in agreement with that of Kearney et al. [26] showing that chlorhexidine retained its activity against these bacteria for 13 and 21 days, respectively. While our data show a sustained bactericidal effect *in vivo* for up to 4 days, irrespective of the frequency of dressing change, we have observed that the *in vitro* drug delivery is that of a "burst" release, the bactericidal efficacy of the chlorhexidine-loaded dressings lasting up to 10 days (unpublished data). However, it is likely that the drug release kinetics from our dressing will be a function of the hydration status of the material. Furthermore, bacterial biofilms can be formed as early as 40 min following contamination of biological surfaces, thereafter becoming harder to eradicate [27]. While this data suggest the need to apply antiseptic-loaded wound dressings to the wounds soon after injury, such wound care strategy is likely impractical in the battlefield due to the hostile environment where the injuries occur. However, Gisby and Bryant [28] have reported that mupirocin cream was effective in reducing the bacterial load of foreign-body induced skin wound infections even when the treatment was delayed by up to 30 h. Similarly, we have shown the effectiveness of liposomal ciprofloxacin-loaded hydrogel dressings in significantly reducing the bacterial load in superficial muscles in a rat model of established wound infection [24]. Although it appears an important consideration that the dressing kills bacteria in the wound very rapidly, it is perhaps more important that the antimicrobial activity be maintained for the wear time of the dressing.

While we evaluated the bactericidal efficacy of our antiseptic-loaded dressings in a porcine model of full-thickness wounds, we elected to determine its wound healing properties on partial-thickness skin defects.

Indeed, the mechanical pressure exerted from the insertion of either of the experimental dressings to completely fill the wounds would have resulted in lesser rates of re-epitheliazation than those observed in the present study [29]. Although the mechanisms for healing partial- and full-thickness wounds differ, the conclusions regarding the effects of the different experimental dressings should remain unchanged. There is also an excellent degree of concordance between human and pig studies investigating the effect of various dressings on the healing of partial-thickness wounds [9]. In agreement with the literature [30-32], the dressing-covered healed faster than those that remained air-exposed. However, the rates of wound healing were quite variable at any given time interval, likely because some of the wounds may have become infected, thereby showing a much slower rate of healing compared to marginally contaminated wounds. As expected, the presence of a hydrogel layer in our dressing contributed to its minimal adherence to the wound bed [33], a desirable property that likely promoted the initially faster rate of wound healing of our dressing compared to that observed when applying Allevyn® by reducing the trauma to the new epithelial layer. Furthermore, dressing adherence to wound bed may lead to pain during its removal. While the control polyurethane foam dressing possesses a non-adherent wound-contacting membrane, it has been shown to induce scab formation on low-exudating wounds such as partial-thickness defects, which will then adhere to the dressing [34]. The latter observation, taken together with our finding of comparable maximum absorption capacities for both dressings (unpublished data), would suggest the greater polyvalence of our dressing.

Immediate cooling of both experimental and clinical burn injuries with cold water has been repeatedly shown to be the most effective way to: reduce skin temperature; reduce the severity of tissue damage; decrease initial local oedema; and, improve wound healing [8, 35-37]. For obvious reasons, this wound care strategy remains impractical in the battlefield. Our finding of a transient decrease in skin temperature following the application of the moist DRDC dressing to rat burns is in agreement with previous studies reporting the cooling properties of hydrogel dressings [36, 38]. However, the magnitude of the change in skin temperature varied greatly depending on the type of hydrogel dressing applied, ranging from approximately 1.5˚C to 4˚C. This discrepancy is likely related to differences in the water content (and thus evaporative cooling capacity) of the various gel formulations. Furthermore, Coats et al. [38] have shown that increasing the air movement over a hydrogel dressing could reduce the skin temperature by up to 10˚C. Interestingly, a large afterdrop in skin temperature after removal of hydrogel dressings was attributed to exposure and subsequent evaporation of volatile substances and water in the residual layer of gel that was left on the skin after removal of the dressings [38]. While excessive cooling of a scald has been shown to be detrimental to wound healing [39], our data show that even a relatively small (1˚C) reduction in skin temperature following application of a hydrogel on the large burn can lead to a significant hypothermia. These negative effects of hydrogels on wound healing and body cooling may contribute to their controversial use on burns.

It is unclear from our data whether the small, short-term reduction in skin temperature that we observed upon application of the moist DRDC dressing would be of clinical relevance. Interestingly, application of a moist prototype DRDC dressing prepared in our laboratories to the forearm of healthy volunteers resulted in a 3˚C reduction in skin temperature for up to 90 min (unpublished data). This data, taken together with the fact that there was there was no effect on skin temperature in the present experiment when applying a dry DRDC dressing on the burn wounds, might suggest that the extent of the decrease in skin temperature is related to the moisture content of the foam layer of the DRDC dressings. It is interesting to speculate that the use of the DRDC dressing on low-exudating wounds (e.g., partial-thickness defects) or on surfaces that require maintenance of their moisture level (e.g., spilled guts) would not reduce their temperature. Nevertheless, care must be taken in interpreting the results from the present experiment in relation to human thermal injury.

5.0 CONCLUSION

These data show that the DRDC dressing is effective in a) delivering medications, such as an antimicrobial agents, to the wound bed; b) promoting faster healing of the treated wound; and c) providing a transient, cooling effect to the skin contact-site, without the adverse effect of inducing whole-body hypothermia like conventional hydrogel wound dressings.

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